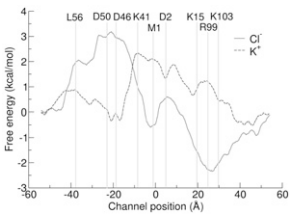
Several recent molecular dynamics simulations only clouded the picture. We carried out rigorous free-energy calculations using all-atom molecular dynamics simulations for Cx26 and Cx32 hemichannels and junctional channels in explicit membrane bilayers. Our potentials of mean force for cation and anion permeation explain the cation selectivity for the Cx26 channel and the modest anion selectivity for the anion selectivity for the Cx32 channel. For Cx26 (see Figure), pore-lining residues K41/M1(N-terminus) and K15/R99/K103 form energy wells for Cl- and barriers for K^+, while D46/D50 form a barrier for Cl- and a well for K^+. For Cx32, E41 forms a barrier for Cl- whereas N2 forms a barrier for K^+. These results provide a solid foundation for quantitatively rationalizing gap junction channel selectivity and conductance. Supported by the National Research Foundation of Korea (2012R1A1A1012707) and KISTI Supercomputing Center (KSC-2011-C2-44), and by NIH Grant GM88187.



2105-Pos Board B124

Dynamics of Water Inside the Secy Translocon Complex

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In bacteria and archea, the insertion of membrane proteins into the plasma membrane is governed by the SecY translocon complex. Understanding how the SecY translocon distinguishes a transmembrane (TM) segment from a secretory one is therefore of the utmost importance. The process of insertion of a TM segment into the membrane resembles a thermodynamic equilibrium process, which has allowed the determination of a ''biological'' hydrophobicity scale [1,2]. Recent measurements have shown that water-to-membrane partitioning is energetically different from translocon-to-membrane partitioning [3]. A possible explanation is the state of water within the translocon and consequently the strength of the hydrophobic effect. In order to shed light on translocon-to-membrane partitioning, we have investigated water dynamics inside the SecY. We performed molecular dynamics simulations using the crystal structure of SecYE from Pyrococcus furiosus [4] as the initial configuration. Approaching the central region of SecY the hydrogen-bond network between water molecules survives longer than that between bulk water molecules. The rotational motion of water molecules is slowed down and the translational dynamics is characterized by ''anomalous'' diffusion. These results might explain the difference between translocon-to-membrane partitioning and water-to-membrane partitioning. The features we observed are characteristic of water molecules located close to a macromolecule. Being affected by the complex shape and the physicochemical heterogeneity of the macromolecular surface, these water molecules manifest different properties from those of the bulk phase. A TM helix passing through the SecY protein-conducting channel will feel an environment different from that characterizing bulk water.

[1] Hessa *et al.*, *Nature*, 377, 433 (2005).
[2] Hessa *et al.*, *Nature*, 1026, 450 (2007).
[3] K. Ö-jemalm *et al.*, *Proc. Natl. Acad. Sci. USA*, E359, 109 (2011).
[4] P. F. Egea and R. M. Stroud, *Proc. Natl. Acad. Sci. USA*, 17182, 107 (2010).

2106-Pos Board B125

Insertion Mechanism in E. Coli of Single-Span Membrane Proteins with Far-Downstream Transmembrane Segments

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Single-span membrane proteins (SSMPs) are the most abundant membrane proteins in virtually all organisms. SSMPs with N-terminus outside the cell are referred to as Type I MPs, while those with the N-terminus inside are referred to as Type II MPs. It is generally assumed that insertion occurs co-translationally via the signal recognition particle (SRP) pathway. The positive-inside rule determines TypeI/II MPs. The TM segment of Type I MPs is generally preceded by a cleavable signal sequence to facilitate the Nout-Cin topology. The TM segment of both types of proteins generally occurs early in the amino acid sequence, allowing recognition by SRP as it emerges from the ribosome. Secreted periplasmic proteins have a signal sequence, but are secreted post-translationally through the translocon by the SecA translocase.
We are studying an unusual class of Type II MPs that lack identifiable signal sequences and whose TM segments can occur hundreds of residues downstream from the N-terminus. One such protein is RodZ, which is a cytoskeletal protein involved in maintaining the rod shape of E. coli. What pathway does this protein use? Several prediction programs and our experimental studies of RodZ with deleted TM segment (RodZ-ΔTM) confirm the absence of a native targeting signal. Proteinase K treatment of spheroplasts reveal that RodZ has Nin-Cout (Type II) topology, consistent with previous studies. Classic N-terminal cleavable signal sequences, such as DsbA or MalE, do not affect the topology of RodZ. We have determined that RodZ insertion is strongly SecA dependent, because RodZ is not incorporated into the membrane under SecA-depletion conditions or in the presence of sodium azide, which is known to inhibit SecA. This result is important, because it is now possible to study the energetics of TM helix insertion by SecA.

2107-Pos Board B126

A Computational Study of H-Ras Nanoclusters in Membrane Domains

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The Ras family of enzymes are guanine triphosphatases (GTPases) that function as molecular switches by cycling between GDP-bound off and GTP-bound on conformational states. Malfunction of Ras proteins due to somatic mutations accounts for about 30% of human tumors. The signaling function of Ras proteins is highly related to their ability to form protein-lipid nanodomains (termed nanoclusters) on the plasma membrane. However, the molecular basis for the formation and distribution of Ras nanoclusters has not been determined. We attempt to address this fundamental issue by studying H-Ras proteins in model membranes and focusing on (1) how multiple Ras proteins oligomerize on the membrane surface, (2) how nanoclustering might be affected by conformational variations, and (3) how nanoclusters might affect the host membrane.
To achieve these goals, we performed coarse-grained molecular dynamics simulations of full-length GDP- and GTP-bound H-Ras in a model membrane. We found that variation in the initial conformation of these two states of H-ras leads to nanoclusters that exhibit different dynamic behaviors. Analysis of protein-protein contacts in the clustered proteins allowed us to map the residues involved in aggregation. By zooming in on the individual residues involved in protein-protein interactions, we found that the two states of H-ras significantly differ in the accessibility and availability of the structural elements that are required for effector binding. Another important observation is that nanoclusters generate positive curvatures on both layers of the membrane. To investigate the mechanisms of these membrane deformations, we performed three dimensional pressure field analyses and determined the surface tension and elastic bending modulus of each monolayer. These results highlight the intricacies of Ras nanocluster formation, which involve both protein-membrane and protein-protein interactions, and pave the way for a better understanding of signal transduction events mediated by Ras clusters.

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Molecular Dynamics Simulations of Caveolin-1 in Membrane Bilayers

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Caveolin which is found in caveolae is an integral membrane protein. Caveolin is thought to induce membrane curvature and is involved in many crucial cell functions such as endocytosis. Its membrane-embedded domain contains two helices (TM1 and TM2) connected by a three-residue linker, and both its N- and C-termini are exposed to the cytoplasm. Since there is no portion of caveolin that protrudes to the opposite side of the membrane, caveolin is postulated to adopt a horseshoe configuration. Despite considerable efforts, the structure of caveolin in a bilayer remains elusive. This work aims to characterize the structure and dynamics of caveolin-1 (D82 to S136; Cav182-136) in a DMPC bilayer using molecular dynamics (MD) simulations. In Cav182-136, TM1 and TM2 corresponds to A87−F107 and L111−A129, respectively. To explore a sufficiently large configurational space of Cav182-136, 50 independent initial models (10 for each of 45°, 55°, 65°, 75°, 86° TM1-TM2 angles) were built and each of them was placed in a DMPC bilayer with a 0.15M KCl solution using the CHARMM-GUI Membrane Builder. The TM1-TM2 linker (G108, I109, and P110) in each of the systems was randomly placed in between Z = −5 to 5 Å. A 50-ns production was performed for each system using CHARMM, and the results are presented and discussed in terms of (1) the orientation of Cav182-136 and its fluctuation [tilt angle of TM1-TM2 plane, TM1-TM2 angle, TM1 and TM2 tilt angle, insertion depth of the TM1-TM2 linker and four Trp residues: 85, 98, 115, 128], (2) TM1-TM2 contact residues, (3) Cav182-136−bilayer interactions, and (4) bilayer shapes.